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A simple and reliable method for the quantitative evaluation of anthocyanin adsorption by wine yeasts

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ABSTRACT

Yeast pigments adsorption can modify wine color intensity and is considered an important trait in wine yeasts. The existing methods for the evaluation of yeast adsorption are laborious, time consuming, need confirmation experiments, or are difficult to apply for a large number of strains generated in breeding programs. In this study, a new test is proposed to measure yeast pigments adsorption and wine color in a single experiment. The optimized method included the microfermentation of grape musts obtained by thermal extraction, digital determination of yeast biomass color, and spectrophotometric wine intensity evaluation. Results showed significant negative correlation between yeast pigments adsorption and wine color intensity. Pigments adsorption occurs from the middle to the end of fermentation, indicating cell wall changes and/or anthocyanins modifications over the process. Significant differences were observed on anthocyanins adsorption and wine intensity among yeast strains independent of the grape variety, and yeasts could be grouped as low, medium and high adsorption strains. The proposed method showed high reproducibility and allows the concomitant screening of hundreds of yeast strains in a short period of time.

1. Introduction

Color intensity and tonality are among the main quality attributes of red wines and a matter of concern of winemakers (Jackson, 2014). The initial color of wines is due to pigments, mainly anthocyanins, extracted from grape skins during the maceration process (Ribereau-Gayon et al., 2006). Anthocyanins in must and wines are present as free (monomeric), polymerized, and *co*-polymerized pigments. Monomeric anthocyanins are water soluble glycosides of anthocyanidins, mainly malvidin-3-O-glucoside, peonidin-3-O-glucoside and petunidin-3-O-glucoside, prevalent in young wines, while polymerized and *co*-polymerized pigments increase during fermentation, maturation, and ageing (He et al., 2012a and He et al., 2012b).

Wine color intensity and tonality are affected by agricultural (grape variety, maturation, vintage, agricultural practices, among others), and enological (extraction system, maceration conditions, alcoholic and malolactic fermentation, yeast strains, ageing) factors. During and after alcoholic fermentation, yeasts can positively contribute for red wine color by: (1) the production of metabolites, like pyruvic acid and acetaldehyde, which can interact with anthocyanin; (2) the release of mannoproteins and other cell wall components, particularly during the autophagic/autolytic process; and (3) the bioconversion of grape precursors generating reactive molecules that interact with anthocyanins increasing pigments polymerization and stability (Morata et al., 2016). However, yeasts can also reduce color intensity and modify wine tonality through deglycosylation of anthocyanins by specific β-glycosidases or anthocyanidases (Manzanares et al., 2000), and the direct adsorption of pigments on yeasts cell surface (Morata et al., 2003, 2005, 2016; Caridi et al., 2007).

The relevance of anthocyanin adsorption by yeasts on wine color has been a matter of discussion for decades due conflicting results obtained by different groups. However, in the last years, its influence on wine color and anthocyanin concentration and stability has been confirmed (Morata et al., 2003, 2005, 2016; Medina et al., 2005; Caridi et al., 2007). Anthocyanin adsorption by yeasts depend on yeast cell wall constitution and the proportion of the different anthocyanins in grape must and wine (Medina et al., 2005; Morata et al., 2003, 2005, 2016). In general, yeast cell wall makes up 15 to 30% of the dry weight of the cell and is mostly composed of mannoproteins, β-1,3-glucans, β-1,6-glucans, and chitin (Lipke and Ovalle, 1998), and its thickness and composition varied among species, strains, and in response to growth conditions (Aguilar-Uscanga and François, 2003).

Yeast pigments adsorption capacity in *Saccharomyces cerevisiae* has been evaluated between 1.6 and 5.8% of total anthocyanins depending on strains (Morata et al., 2005), and exhibits a polygenic inheritance (Caridi et al., 2007). In this context, yeast anthocyanins adsorption is

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pointed out as an important trait on yeast selection and improvement for wine production (Pretorius, 2000; Fleet, 2008; Bisson and Karpel, 2010).

Several methods have been proposed for the evaluation of yeast pigments adsorption capacity. Morata et al. (2003) analyzed yeast adsorption by a method that involves red wine fermentation, anthocyanins extraction from yeast cells, and anthocyanins analysis by HPLC-DAD-MS. Although time consuming and not appropriate for large number of samples, this method is considered as standard by the International Vine and Wine Organization (OIV, 2012). Medina et al. (2005) developed a model red grape juice for the indirect determination of yeast adsorption by the analysis of wine anthocyanins using HPLC-DAD. To evaluate a higher number of yeast stains, Caridi, 2006, Caridi, 2013, Caridi et al., 2015 proposed a method that involves yeast growth on a solid "grape skin agar" medium on Petri dishes, evaluating their adsorption capacity (WCA- wine color adsorption) by photographing yeast biomass and measuring their red, green, and blue color intensity by a computing-assisted evaluation.

Although efficient, these methods have several limitations for the analysis of the large number of yeasts strains generated in breeding program, the evaluation of yeast adsorption in fermentative conditions, the analysis of yeast strains adsorption capacity on must from different grape varieties, and the concomitant evaluation of yeast adsorption and its effect on wine color. To solve these limitations, we developed and optimize a simple and reliable method that involves microvinification followed by yeast adsorption and wine color analyses that can be applied for the evaluation of hundreds of strains at a time in a short period.

2. Materials and methods

2.1. Yeast strains and growth conditions

Twenty two commercial yeast strains (*Saccharomyces cerevisiae*) EC1118, QA23, QD145, CY3079, Cross Evolution, Elegance (Lallemand, Canada); Maurivin B, AWRI-R2, AWRI-350, AWRI-796, BP725, UCD522, Pris de Mousse (PDM) (Maurivin, Australia); Vl-1, VL2, VL-5, Spark, F33, F15 (Laffort, France), Y904 (COATEC, Brazil); Rouge (Fermol, Italy), Red Fruit (Enartis, Italy), and three *S. cerevisiae* (MPF, LAMF, LACF) and one *Torulaspora delbrueckii* (TPI-4) isolated from Brazilian vineyard, were used in the experiments.

YEPD broth (1% yeast extract, 2% peptone, 2% glucose), or solidified with 2% agar when required, was used for yeast maintenance and multiplication.

Yeast cell enumeration for inoculum determination and the evaluation of yeast population during alcoholic fermentation was carried out by microscopic cell counting using a Neubauer chamber.

2.2. Microfermentations

Small scale fermentations were undertaken using *Vitis vinifera* vr. Merlot and Cabernet Sauvignon, and *Vitis labrusca* vr. Ives musts. Musts were obtained by liquefying the berries (3 pulses of 30 s), heating at 70 °C for 30 min, filtering through cheesecloth, and autoclaving at 121 °C for 30 min. Musts were conserved under refrigeration (4 °C) in the dark. Previous to the experiments musts were centrifuged (6000 ×*g*, 10 min) and filter (45 μm) to remove particles. Yeasts were inoculated (1–2 × 10^7 cels/ml) from a pre-culture of 48 h at 25 °C in YEPD.

Fermentations (800 μl) were conducted in 96 deep wells storage plates with 2.2 ml capacity (Scientific Specialties Inc., USA) sealed with Platemax sealing films (Axygen, USA). Fermentations were maintained stationary at 25 °C, and vortexed (IKA MS3 vortexer, Germany) once a day (1 min, 1500 rpm) to increase cell/must contact.

At the end of fermentation (14 days), plates were centrifuged at 3600 rpm, 4 °C, for 5 min. Wines (50 μl) were transferred to 96 wells Elisa-plates (TPP, Swirzerland) for the evaluation of wine color intensity and tonality, while cell pellets were washed twice with water, and suspended in 200 μl of 10% ethanol solution in 20 mM tartrate buffer (pH 3.6) for the analysis of yeast pigments adsorption.

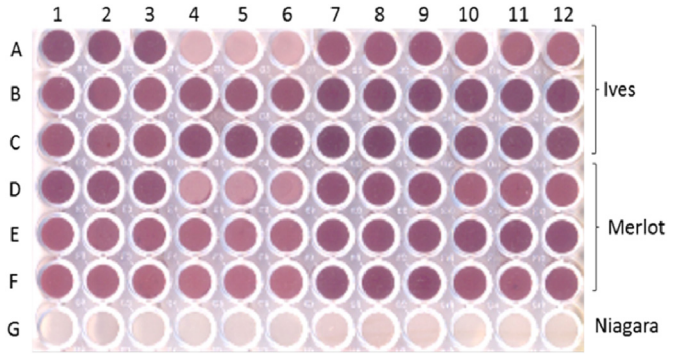

Fig. 1. An example of an yeast pigments adsorption plate showing high reproducibility, and variation among yeast strains in musts from two grape varieties (Ives – *V. labrusca*, and Merlot – *V. vinifera*), and yeast background color in a white must (Niagara). Yeast strains (columns; rows): Red Fruit (1–3; A,D,G), Rouge (4–6; A,D,G), Y904 (7–9; A,D,G), QA23 (10–12; A,D,G), EC1118 (1–3; B,E), UCD522 (4–6; B,E), F15 (7–9; B,E), Spark (10–12; B,E), BP725 (1–3; C,F), Elegance(4–6; C,F), TPI-4 (7–9; C,F), CY3079 (10–12; C,F). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. Wine color density and tint

Wine samples were diluted (1:4) with a 10% ethanol solution in 20 mM tartrate buffer (pH 3.6) (150 μl), and evaluated in an ASYS Expert Plus microplate reader (Biochrom, UK). Absorbance at 420, 520, and 620 nm were used to calculate wine color intensity ($A_{420} + A_{520} + A_{620}$), and color hue ($A_{420}/A_{520}$). Using the 1:4 dilution the adsorption values varied between 0.3 and 0.9.

2.4. Yeast pigments adsorption

Washed yeast cell suspensions (200 μl) were transferred to 96 wells Elisa-plates (TPP, Switzerland) and centrifuged at 3600 rpm, 4 °C, for 5 min. The plates (Fig. 1) were digitalized using a scanner, and analyzed with Adobe Photoshop CS2 Version 9.0 for Windows. Each spot region was delimited and their red (R), green (G), blue (B), and mean RGB values computed. Each value, ranging from 0 (black) to 255 (white), was used to calculate Relative Yeast Pigments Adsorption [RYPA = −(RGB control – RGB yeast sample]. RGB control is the mean value of tree independent non inoculated must samples processed and analyzed in the same way as the fermented ones. RGB control values varied between 245 and 251 among experiments (almost white), indicating very low interference of residual colored particles. Moreover, yeasts samples from white must fermentation showed RGB values of 252–254, indicating noninterference of yeast cell background on pigments adsorption determination (Fig. 1).

2.5. Statistical analysis

Descriptive parameters (means and standard deviations), One-Way ANOVA, means comparison (Tukey's HSD test, $p < .05$), and Pearson correlations were performed using the IBM SPSS 20.0 Statistics program for Windows.

3. Results and discussion

In order to determine the effect of different parameters and optimize the evaluation of yeast pigments adsorption, wine intensity and tint by

the proposed method, we developed several experiments using *S. cerevisiae* Red Fruit and F33, and a Merlot must. Yeast strains were selected based on their high and medium adsorption capacity, respectively, and Merlot must considering variety importance in Brazilian red wine production.

A first experiment was designed to evaluate the effect of ethanol and pH on wine and pigments adsorption values. Thus for, distilled water, PBS (10 mM phosphate buffer, pH 7.0), 10% ethanol (pH 7.0), and 10% ethanol (20 mM tartrate buffer, pH 3.6) were used to dilute wine samples, and to wash and suspend yeast cells. The results (data not shown) indicated that 10% ethanol (pH 3.6) maintained wine color intensity and tint, and increase the red adsorption by yeast cells when compared with water, PBS and ethanol (pH 7.0). Low pH and ethanol guarantee the equilibrium between red (flavilium cations), blue (quinoidal) and yellow/colorless (chalcone) forms of anthocyanins currently found in wines (He et al., 2012a). Low pH and 10–12% ethanol has been used to evaluate wine color and the determination of the chromogenic characteristic of wines (Pérez-Caballero et al., 2003).

In order to evaluate the effect of cell number per sample on the relative adsorption data, a serial dilution of Red Fruit and F33 yeast cells obtained after fermentation (14 days) of a Merlot must were deposited on Elisa plates and evaluated. The results (Fig. 2A) showed that independent of their differences on pigments adsorption capacity, both strains exhibited constant relative adsorption (RYPA) values above 5×10^7 cells/sample (200 µl). This fact can be attributed to the formation of a homogenous cell layer in high cell densities reducing light interference by transparence during scanning. Under wine fermentation conditions, wine yeast strains of *Saccharomyces* reach between 0.8 and 2.5×10^8 cells/ml, such that a fermentation of 800 µl is sufficient to generate the number of cells necessary for the determination of yeast pigments adsorption.

To determine yeast pigments adsorption along wine fermentation, 400 ml experiment was conducted with Red Fruit strain on Merlot must collecting samples at different intervals and standardizing yeast cell number per sample (2×10^8 cell/200 µl) for the evaluation of RGB values. As can be observed in Fig. 2B, yeast pigments adsorption started after 6 days, three days after yeast population reached stationary phase, and increased until day 11, remaining stable on the following days. Based on these results yeast adsorption capacity should be evaluated at the end of fermentation, that correspond to 12 to 15 days under the experimental conditions. Yeast adsorption behavior during wine fermentation may be associated with the increase of acylated anthocyanins during fermentation, as these derivatives are more strongly adsorbed by yeasts (Morata et al., 2003; Medina et al., 2005; 2018), and the modification of yeast cell wall composition and affinity (Aguilar-Uscanga and François, 2003).

The image analysis of yeast cells allow to computed the red (R), green (G), and blue (B) colors, and a RGB mean of the samples. To define the relation between these data and the wine color intensity, an experiment with fifteen wine yeast strains fermenting a Merlot must was developed. The results (Fig. 3A) showed that the mean of RGB can be used as a general "relative adsorption" value, as it highly correlated with Red ($R = 0.88$), Green ($R = 0.92$), and Blue ($R = 0.73$) individual mean values. Moreover, as can be observed in Fig. 3B, the R, G, B, and RGB values significantly correlated with wine color intensity ($R = -0.897$), confirming the role of yeast anthocyanin adsorption on the reduction of wine color during the fermentation process (Morata et al., 2003, 2005; Morata et al., 2016). It is worth pointing out that while red and green values are almost linear all along the distribution, blue values had a linear behavior just in low and middle adsorption strains.

Based on the above results, twenty commercial wine yeast strains were evaluated on three musts obtained from two *Vitis vinifera* (Mertot and Cabernet Sauvignon) and a *Vitis labrusca* (Ives) varieties, to determine the effect of grape variety on the adsorption capacity and its relation with wine color intensity. The results (Table 1) showed significant variation of anthocyanins adsorption and wine color intensity among yeast strains and among grape varieties. However, interaction between yeast strains and grape varieties was not-significant, and yeast strains grouped as low, medium and high adsorption capacity were constant. Thus, independent of grape must, yeast strains with lowest adsorption were Rouge, VL-1, VL-2, VL-5, AWRI-R2, and F33, and those with high adsorption were TPI-4 (*T. delbrueckii*), Y904, Red Fruit, Elegance, AWRI-350, and CY3079. Regarding wine tint, significant differences were observed between grape varieties, but no significant differences were detected between yeast strains. (Caridi, 2013) reported significant interaction between yeast adsorption and must origin (variety). The disagreement between these results can be attributed to differences on varietal anthocyanins composition between the varieties used in each evaluation (Pomar et al., 2005), yeast strains analyzed that varied on their pigments versus cell wall mannoproteins interactions capacities (Caridi, 2006). Independent of this fact, the present method allows the evaluation of yeast adsorption, and its effect on wines obtained from musts from different origins and varieties, allowing the best experimental configuration for the intended purpose.

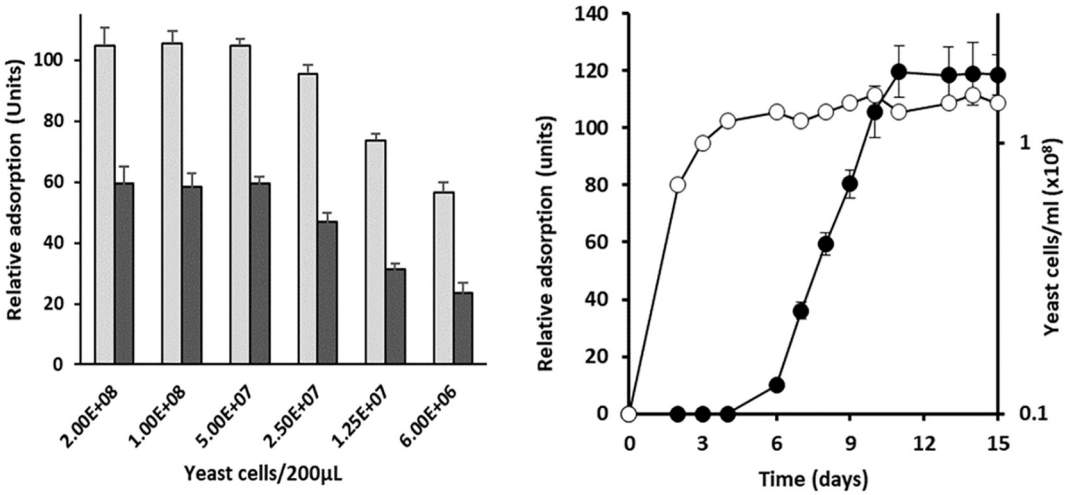

Fig. 2. Two main factors that influence the evaluation of relative pigments adsorption: (A) the effect of cell number per sample (A): Red Fruit (light grey) and F33 (dark grey), and (B) the effect of yeast population (open circles) and yeast pigments adsorption (close circles) along wine fermentation. Data represent means and standard deviations from three replications. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

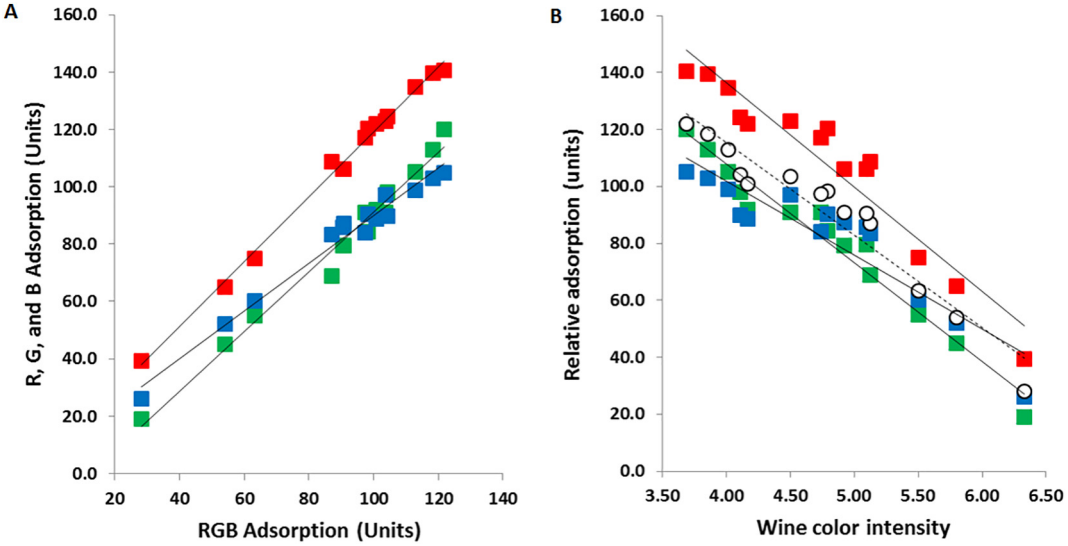

Fig. 3. Relation between (A) red (red squares), green (green squares), blue (blue squares) values and RGB means, and (B) between red, green, blue, and RGB (open circles) and wine color intensity. Data are mean values of twelve commercial wine yeast strains. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Color intensity and relative pigments adsorption (means and standard deviations) of twenty commercial wine yeast strains on musts from three red grape varieties.

	Ives		Merlot		Cabernet Sauvignon	
	Intensity	Adsorption	Intensity	Adsorption	Intensity	Adsorption
Red Fruit	6.11 ± 0.04^{e}	102.8 ± 0.57^{de}	5.25 ± 0.16^{d}	100.9 ± 1.5^{a}	3.46 ± 0.04^{ab}	90.7 ± 0.9^{bc}
Rouge	8.13 ± 0.04^{a}	28.2 ± 3.1^{i}	6.86 ± 0.03^{a}	51.7 ± 5.9^{e}	3.71 ± 0.09^{a}	30.4 ± 2.3^{e}
Maurivin B	6.16 ± 0.03^{e}	97.6 ± 2.7^{ef}	5.08 ± 0.28^{de}	105.7 ± 1.2^{a}	3.19 ± 0.23^{b}	110.2 ± 1.5^{a}
QA23	7.12 ± 0.12^{b}	87.0 ± 2.6^{g}	5.75 ± 0.04^{c}	92.0 ± 1.0^{b}	3.37 ± 0.06^{ab}	85.1 ± 2.8^{bc}
EC1118	7.09 ± 0.22^{bc}	93.9 ± 1.9^{f}	6.06 ± 0.09^{bc}	88.5 ± 1.9^{bc}	3.41 ± 0.12^{ab}	95.7 ± 0.7^{b}
UCD522	6.79 ± 0.12^{cd}	98.3 ± 0.9^{ef}	6.22 ± 0.05^{b}	82.7 ± 2.5^{cd}	3.60 ± 0.07^{a}	71.8 ± 3.6^{d}
AWRI-350	6.01 ± 0.12^{e}	112.9 ± 1.8^{b}	5.28 ± 0.14^{d}	103.5 ± 0.5^{a}	3.40 ± 0.10^{ab}	80.1 ± 1.6^{cd}
Y904	5.69 ± 0.09^{f}	109.8 ± 0.5^{bc}	4.98 ± 0.15^{de}	106.1 ± 0.3^{a}	3.13 ± 0.04^{b}	88.5 ± 2.6^{bc}
BP725	6.92 ± 0.02^{bcd}	94.6 ± 2.0^{f}	6.04 ± 0.11^{bc}	85.8 ± 0.6^{bc}	3.41 ± 0.26^{ab}	95.9 ± 1.6^{b}
Elegance	6.74 ± 0.10^{d}	106.3 ± 0.9^{cd}	6.23 ± 0.08^{b}	76.1 ± 1.1^{d}	3.64 ± 0.16^{a}	71.2 ± 8.8^{d}
TPI4	5.85 ± 0.12^{ef}	118.4 ± 0.7^{a}	4.85 ± 0.07^{e}	105.4 ± 1.1^{a}	3.08 ± 0.13^{b}	110.1 ± 4.6^{a}
VL-5	8.04 ± 0.04^{a}	31.4 ± 2.1^{i}	6.72 ± 0.03^{a}	54.6 ± 4.2^{e}	3.68 ± 0.12^{a}	32.2 ± 1.9^{e}
VL-1	7.89 ± 0.11^{a}	45.7 ± 4.3^{h}	6.44 ± 0.09^{a}	65.2 ± 3.8^{e}	3.49 ± 0.10^{a}	39.6 ± 2.2^{e}
VL-2	8.12 ± 0.05^{a}	30.5 ± 2.5^{i}	6.97 ± 0.08^{a}	52.2 ± 3.6^{e}	3.84 ± 0.09^{a}	29.7 ± 1.5^{e}
F15	6.97 ± 0.13^{b}	87.4 ± 2.2^{g}	5.62 ± 0.07^{c}	95.8 ± 0.8^{b}	3.26 ± 0.06^{ab}	88.2 ± 2.5^{bc}
F33	7.77 ± 0.09^{a}	49.4 ± 1.7^{hi}	6.36 ± 0.03^{ab}	58.6 ± 2.4^{e}	3.54 ± 0.12^{ab}	39.9 ± 2.0^{e}
AWRI-R2	7.83 ± 0.12^{a}	38.0 ± 1.1^{i}	6.55 ± 0.06^{ab}	56.2 ± 3.2^{e}	3.58 ± 0.10^{ab}	34.4 ± 1.8^{e}
AWRI-796	6.81 ± 0.07^{cd}	99.2 ± 2.7^{f}	6.15 ± 0.22^{de}	102.7 ± 1.4^{a}	3.19 ± 0.11^{b}	113.2 ± 1.7^{a}
Spark	7.27 ± 0.21^{bc}	97.2 ± 0.9^{ef}	6.30 ± 0.13^{bc}	89.5 ± 1.9^{bc}	3.52 ± 0.12^{ab}	98.3 ± 0.8^{b}
CY3079	6.94 ± 0.14^{bcd}	103.3 ± 0.9^{cd}	6.33 ± 0.10^{b}	97.3 ± 1.1^{ab}	3.66 ± 0.16^{a}	101.2 ± 5.6^{ab}

Different letters within a column indicate significant differences at 95% of confidence level by the Tukey's HSD test.

4. Conclusion

This work described a simple and optimized method for the monitoring of yeast pigments adsorption and wine color changes using microvinification, spectrophotometric determination of wine color intensity, and digital determination of yeast relative pigments adsorption in a single experiments. The method can be used for the evaluation of high number of yeast strains and species, and designed for any intended purpose (must origin, yeast strain, breeding). The method confirmed the variation on yeast adsorption capacity, and its interaction with grape varieties anthocyanin composition. Moreover, data showed a particular behavior on yeast adsorption, with high pigments retention through the final stages of fermentation.

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References

Aguilar-Uscanga, B., François, J.M., 2003. A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. Lett. Appl. Microbiol. 37, 266–274.
Bisson, L., Karpel, J.E., 2010. Genetics of yeast impacting wine quality. Ann. Rev. Food Sci. Technol. 1, 139–162.
Caridi, A., 2006. Enological functions of parietal yeast mannoproteins. Anton. Van Leeuwenhoek 89, 417–422.
Caridi, A., 2013. Improved screening method for the selection of wine yeasts based on their pigment adsorption activity. Food Technol. Biotechnol. 51, 137–144.
Caridi, A., Sidari, R., Solieri, L., Cufari, A., Giudici, P., 2007. Wine colour adsorption phenotype: an inheritable quantitative trait loci of yeasts. J. Appl. Microbiol. 103, 735–742.
Caridi, A., Sidari, R., Krakova, L., Kuchta, T., Pangallo, D., 2015. Assessment of color adsorption by yeasts using grape skin agar and impact on red wine color. J. Int. Sci. Vigne Vin. 49, 195–203.

Fleet, G.H., 2008. Wine yeasts for the future. FEMS Yeast Res. 8, 979–995.
He, F., Liang, N.N., Mu, L., Pan, Q.H., Wang, J., Reeves, M.J., Duan, C.Q., 2012a. Anthocyanins and their variation in red wines. I. Monomeric anthocyanins and their color expression. Molecules 17, 1571–1601.
He, F., Liang, N.N., Mu, L., Pan, Q.H., Wang, J., Reeves, M.J., Duan, C.Q., 2012b. Anthocyanins and their variation in red wines. II. Anthocyanin derived pigments and their color evolution. Molecules 17, 1483–1519.
Jackson, R.S., 2014. Sensory perception and wine assessment. Chapter 11 In: Wine Science: Principles and Applications, 4th Edition. Academic Press, Elsevier (933 p).
Lipke, P.N., Ovalle, R., 1998. Cell wall architecture in yeast: new structure and new challenges. J. Bact. 180, 3735–3740.
Manzanares, P., Rojas, V., Genovés, S., Vallés, S., 2000. A preliminar search for anthocyanin-β-D-glucosidase activity in non-*Saccharomyces* wine yeasts. Int. J. Food Sci. Techn. 35, 95–103.
Medina, K., Boido, E., Dellacassa, E., Carrau, F., 2005. Yeast interactions with anthocyanins during red wine fermentation. Am. J. Enol. Vitic. 56, 104–109.
Medina, K., Boido, E., Dellacassa, E., Carrau, F., 2018. Effects of non-*Saccharomyces* yeasts on color, anthocyanin and anthocyanin-derived pigments of Tannat grapes during fermentation. Am. J. Enol. Vitic. 69, 148–156.
Morata, A., Gómez-Cordovés, M.C., Suberviola, J., Bartolomé, B., Colombo, B., Suárez, J.A., 2003. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. J. Agric. Food Chem. 51, 4084–4088.
Morata, A., Gómez-Cordovés, M.C., Colombo, B., Suárez, J.A., 2005. Cell wall anthocyanin adsorption by different *Saccharomyces* strains during the fermentation of *Vitis vinifera* L. cv. Graciano grapes. Eur. Food Res. Technol. 220, 341–346.
Morata, A., Iris, L., Suárez, J.A., 2016. Influence of yeasts in wine colour. Chapter 13 In: Morata, A., Loira, I. (Eds.), Grape and Wine Biotechnology. IntechOpen, pp. 285–305.
OIV (2012) Guidelines for the characterization of wine yeasts of the genus *Saccharomyces* isolated from vitivinicultural environments: low cell wall anthocyanin adsorption. Resolution OIV-Oeno 370–2012.
Pérez-Caballero, V., Ayala, F., Echávarri, J.R., Negueruela, A.I., 2003. Proposal for a new standard OIV method for determination of chromatic characteristics of wine. Am. J. Enol. Vitic. 54, 59–62.
Pomar, F., Novo, M., Masa, A., 2005. Varietal differences among the anthocyanin profiles of 50 red table grape cultivars studied by high performance liquid chromatography. J. Chromat. A 1094, 34–41.
Pretorius, I.S., 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16, 675–729.
Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A., 2006. Handbook of Enology. Vol. 1. The microbiology of wine and vinifications, 2nd. John Wiley & Sons, England, pp. 497.